Hypoallergenic Breads

Wheat Content of Products Available in the San Francisco Bay Area

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■ *Substantial amounts of soluble wheat antigens have been found in breads sold as "wheat-free" in the San Francisco Bay Area. Physicians with patients on a wheat-free diet are urged to exercise careful supervision over their patients' choice of such breads.*

FOR THE STUDY AND CONTROL of allergic sensitivity to wheat in humans total elimination of this grain from the diet is necessary. In addition, the initial elimination of other cereal grains is important because of their varying content of proteins identical to those in wheat and the presence of antibodies to proteins in such cereals cross reacting with wheat in the patient's serum. Breads and other bakery products made of soy or lima bean flour, potato starch, a corn starch-free baking powder, salt and water, according to our recipes[4] in the home or by cooperative bakers, have long been used for such study in our practice.

Serious asthma or other clinical allergic disease in highly wheat-sensitive persons has occurred following the ingestion of commercial bakery products containing gluten or other wheat proteins but not so labeled. The substitution of home-made or accurately prepared commercial bakery products found by us to contain no wheat protein has resulted in the control of such wheat sensitivity disease.

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Materials and Methods

To determine unlisted wheat proteins in bakery or other food products, sample loaves of bread were purchased at 14 health food stores in five counties of the San Francisco area. For purposes of this study "bread" is considered to include such products as hamburger buns but not to include cinnamon rolls or the like. A sample of each loaf purchased was allowed to dry in the air at room temperature. One gram of each dry sample was then extracted for 24 hours at 4°C with 10 ml of isotonic saline solution.

Standard solutions of wheat antigens were prepared by similarly extracting 1 gm of wheat flour, commercial gluten flour and gluten* with 10 ml of saline solution each.

Reagents

Saline. Nine volumes of sodium chloride solution, 8.8 gm per liter, were mixed with 1 volume of phosphate buffer, 32.7 gm $Na_2HPO_4.7H_2O$ and 4.0 gm KH_2PO_4 per liter.

Antibody. Four rabbits were each injected intramuscularly with 1 ml of standard wheat flour suspension weekly for seven weeks, rested five weeks and reinjected. One week later they were

*Nutritional Biochemicals Corporation, Cleveland, Ohio.

bled and the serum obtained. The highest titer serum, as judged by precipitin tests, was used for these experiments.

Antibody Specificity

These sera contained no antibody to materials other than wheat or rye which might legitimately be present in these breads. Other cereal grains tested, with completely negative results, include extracts of oats, corn (Zea mays seed), rice, millet and wild rice. Barley, rye and sorghum seed extracts yielded lines with these sera but only the reactions with barley were strong enough to cause confusion, the rye and sorghum reactions being very weak. Authentic 100 per cent rye bread gave a reading of 3 per cent "wheat flour" by this technique. As may be seen, however, from Figure 1, the slope of the calibration curve becomes so steep below 10 per cent wheat flour that figures of less than 10 per cent are almost meaningless. In any case no labels on any of these breads implied in any way the presence of barley or sorghum, which must therefore be considered adulterants if they should be present. The conclusive check on specificity of the anti-wheat sera (at least for this purpose) is that authentic samples of wheat-free bread which contained the same ingredients as were listed on the contaminated breads yielded no Ouchterlony or radial diffusion bands with the sera.

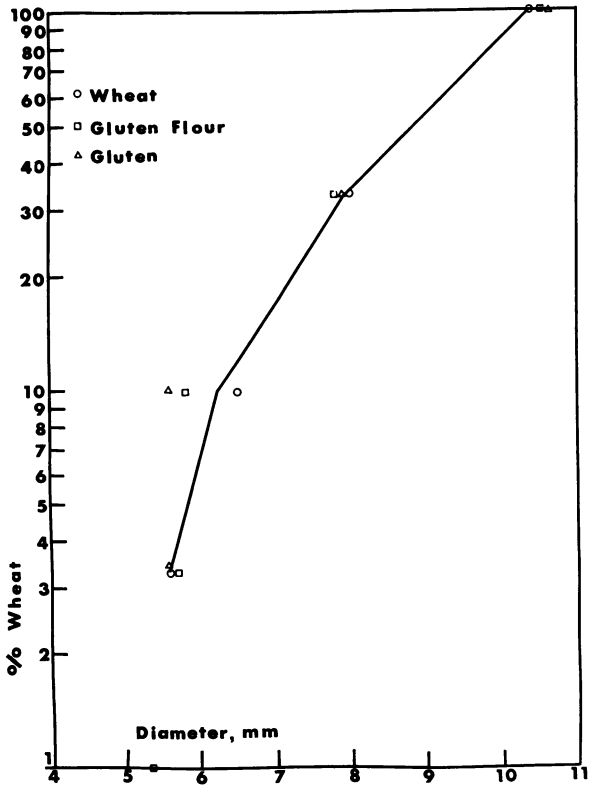

Figure 1.—Radial Diffusion Analyses, Standard Curve. Soluble antigens in bread expressed as per cent wheat flour, *versus* diameters of circles of antigen-antibody precipitates. Standard antigens: wheat flour, gluten flour and gluten (NBC).

Radial Diffusion Plates

For carrying out analyses by a modification of the radial immunodiffusion technique of Mancini and coworkers,[1,2] vessels were prepared by cementing 9 cm diameter plastic rings to a pane of glass. To 5 ml of 3 per cent agar at 60°C was added 5 ml of rabbit antiserum to wheat at 50°C. After thorough mixing, the solution was poured into one of the radial diffusion vessels. When the gel had hardened, holes 4 mm in diameter and 12 mm between centers were punched in it in a hexagonal pattern. Each sample extract was used to fill one of these wells. It is important that the meniscus be neither convex nor concave but as flat as possible. The standard solutions were each diluted 1:3, 1:10, 1:30, 1:100 and 1:300, and each dilution was used to fill one well.

The plate was put into a humid chamber at room temperature and allowed to develop for 24 hours. At this time the diameters of the circles of precipitate around each hole were measured and recorded. The plate was then washed with five changes of saline solution, 24 hours at room temperature for each wash. After the last wash, the plate was stained with 1 per cent Buffalo black in 5 per cent acetic acid for 30 minutes. The stained plates were then washed repeatedly in 2.5 per cent acetic acid until the excess dye was removed. Final readings of the diameters were then taken (Figure 1).

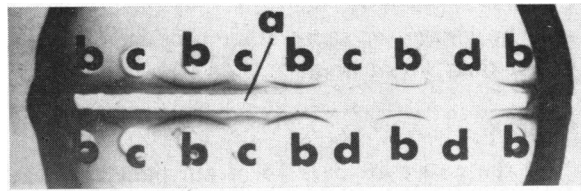

Figure 2.—Typical Ouchterlony Plate. The contents of the wells are: a. Antibody to wheat (in trough); b. Extract of known wheat; c. Extracts from "wheat-free" breads containing 10 per cent or more wheat flour by radial diffusion measurements; d. Extracts from wheat-free breads containing no significant amount of wheat flour by radial diffusion measurements.

TABLE 1. — *Measurements of soluble wheat antigens, as percentage content of wheat flour in "wheat-free" breads**

Soy-Potato and Lima-Potato Breads (10 loaves)	*Other "Wheat-Free" Breads (Rice, Millet, Rye, etc.) (9 loaves)*
72 per cent	48 per cent
37	34
25	17
22	16
19	15
14	9
13	3
11	0
0	0
0	

*Since it can not be established in what form (wheat flour, high-protein wheat flour, gluten flour, etc.) the soluble wheat antigens became incorporated in the bread, the results are expressed as percentage content, on a dry weight basis, of wheat flour which would be necessary to produce the readings obtained.

Ouchterlony Plates

Vessels made by cementing plastic rings to a pane of glass were also used for double diffusion studies by a modification of the method of Ouchterlony[3] (Figure 2).

Results

Laboratory

Percentages of wheat flour found in breads, as calculated from measured ring sizes by use of the standardization curve of Figure 1, are shown in Table 1. Of ten loaves of soy-potato and lima-potato bread, eight contained soluble wheat antigens corresponding to more than 10 per cent wheat flour in the bread, up to a maximum of 72 per cent. Of nine loaves of other breads labeled and sold as "wheat-free," five contained soluble wheat antigens corresponding to more than 10 per cent wheat flour in the bread.

Ouchterlony plate results (Figure 2) confirmed the occurrence of wheat antigens demonstrated by the semi-quantitative radial diffusion method. The absence of one wheat antigen in these breads in the presence of other wheat antigens, as shown by lines of identity, suggests that the adulterant may be gluten, or some other product of wheat, rather than wheat flour.

Clinical

In the past two years 15 of our patients whose symptoms were well controlled by the elimination of wheat and other foods had reactivation of clinical allergic symptoms after eating a commercial bread sold as being wheat-free. Wheat was demonstrated in these breads by the immunological techniques described in this article. When bread and bakery products shown to be wheat-free in our laboratory were resumed, the symptoms were controlled in four to eight days.

Discussion

The radial diffusion technique used in the present study can properly be used for exact quantitation only if the antiserum is monospecific for one and only one antigen in the test extracts. We have been unwilling to adhere to this requirement, and hence the percentages of wheat flour reported here can be considered only as guides to the extent of adulteration rather than as high precision measurements.

When an antiserum reacting with several substances in the wheat is used, each substance in the wheat can be postulated to react separately with its own antibody, resulting in not one ring around the antigen well, but in several concentric rings. It then becomes necessary to select one ring in particular for the measurements and use only it. For this work we selected the outermost ring, relying on pattern recognition to ensure that it corresponded to the same antigen in every case. Fortuitously, calibration curves with wheat flour, gluten flour and gluten were indistinguishable on this basis (Figure 1).

Any errors possible result from breakdown of the postulate of separate antigen-antibody reactions and from errors in pattern recognition. These causes can lead neither to finding wheat where it does not exist nor to gross errors in measurements. If a sample of bread were contaminated with some wheat product (for example, gluten, gluten flour or wheat starch) which did not contain the usual proportion of the antigen normally present in the outer ring, the next ring in would become the outer ring, and if it were read as the outer ring, the per cent wheat flour equivalent found would be too low—that is, errors from this source would cause the bread to appear less contaminated than it really was.

If a monospecific antiserum were used, it could detect only one of the many antigens present in wheat. Adulteration with a wheat preparation (such as gluten) which did not contain this one antigen could then not be detected with this serum. That situation would be tolerable if all wheat-sensitive patients were sensitive only to one and the same antigen. We have evidence that, unfortunately, this is not the case. We have found anti-

bodies in sera of wheat-sensitive and food-sensitive patients to antigens as diverse as gluten, wheat globulins and even wheat starch. It is entirely possible, therefore, that of the various soluble antigens detectable by this technique, some patients are sensitive to one, and other patients to others. For this reason we elected to use wheat antiserum reacting to many wheat antigens and to forfeit the higher precision attainable by use of monospecific antibody.

Our findings are quoted to two significant figures (of which the last figure is uncertain, in accordance with standard practice) only. For establishing whether or not the contaminant is a trace contaminant or a major adulterant, this is sufficient precision. In order to avoid implying more precision than exists, figures are given as percentages of wheat flour equivalent present in the bread rather than as milligrams of antigen per milliliter.

Clinical

The presence of large amounts of wheat in commercial "wheat-free" breads has been demonstrated by radial diffusion, by Ouchterlony tests, and by clinical responses of wheat-sensitive patients eating them.

Both medical profession and lay public must realize that bakery and other food products sold as being wheat-free may contain gluten and other wheat proteins in spite of labeling to the contrary. The ingestion of such products by wheat-sensitive patients will frequently reactivate or perpetuate symptoms due to wheat allergy. Furthermore, the unsuspected ingestion of gluten* or other wheat proteins in these products will prevent the relief of coeliac disease and nontropical sprue when gluten is the cause.

There is no objection to adding wheat proteins, including gluten, to food products provided such proteins are printed on the label and the products are not sold as being wheat-free.

The physician can suspect the presence of wheat in a commercial bakery or food product if clinical symptoms arise after the ingestion of the product by a symptom-free wheat-sensitive patient.

It can also be determined by our immunological technique for the estimation of wheat proteins in food products. This technique can also be used to determine proteins of other foods in commercial products.

These studies emphasize the necessity not only of accurate labeling, but also of listing every ingredient in commercial bakery and other food products. To be certain that such products contain no wheat, patients should make them at home according to prescribed wheat-free recipes or should buy them from bakers who are making them by such recipes and who are supervised by the physician.

*This technique is incapable of showing pure gluten, which is insoluble in saline solution. However, all commercial gluten we have examined has soluble wheat proteins contaminating it (Figure 1).

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